



## AIB1, A NOVEL STEROID RECEPTOR CO-ACTIVATOR

### PRIORITY CLAIM

This application claims priority under 35 U.S.C. § 119 to PCT Application No.

- 5 PCT/US8912689, filed June 17, 1998, which claims priority to U.S. Provisional Application No. 60/049,728, filed June 17, 1997.

### **BACKGROUND OF THE INVENTION**

10 Breast cancer arises from estrogen-responsive breast epithelial cells. Estrogen activity is thought to promote the development of breast cancer, and many breast cancers are initially dependent on estrogen at the time of diagnosis. Anti-estrogen compositions have therefore been used to treat breast cancer.

A frequent mechanism of increased gene expression in human cancers is amplification, i.e., the copy number of a DNA sequence is increased, in a cancer cell compared to a non-cancerous cell. In breast cancer, commonly amplified regions are derived from 17q21, 8q24, and 11q13 which encode erbB-2, c-myc, and cyclic D1 respectively (Devilee et al., 1994, Crit. Rev. Oncog. 5:247-270). Recently, molecular cytogenetic studies have revealed the occurrence in breast cancers of additional regions of increased DNA copy number (Isola et al., Am. J. Pathol. 147:905-911, 1995; Kallioniemi et al., Proc. Natl. Acad. Sci. USA 91:2156-2160, 1994; Muleris et al., Genes Chromo. Cancer 10:160-170, 1994; Tanner et al., Cancer Research 54:4257-4260, 1994; Guan et al., Nat. Genet. 8:155-161, 1994).

Breast cancer is the second leading cause of cancer deaths in American women, and it is estimated that an American woman has at least a 10% cumulative lifetime risk of developing this disease. Early diagnosis is an important factor in breast cancer prognosis and affects not only survival rate, but the range of therapeutic options available to the patient. For instance, if diagnosed early, a "lumpectomy" may be performed, whereas later diagnosis tends to be associated with more invasive and traumatic surgical treatments such as radical mastectomy. The treatment of other cancers likewise is benefitted by early diagnosis, for instance the prognosis in the treatment of lung cancer, colorectal cancer and prostate cancers is greatly improved by early diagnosis. There is a need for a simple and reliable method of diagnosis of cancers in general and of breast cancer in particular. There is a need for a method of screening for compounds that inhibit the interaction between an estrogen receptor ER and an ER-dependent nuclear receptor co-activator molecule in order to identify molecules useful in research diagnosis and treatment of cancer. There is also a need for a method for identifying tamoxifen-sensitive cancer patients in order to better manage treatment. A solution to these needs would improve cancer treatment and research and would save lives.

### **SUMMARY OF THE INVENTION**

40 The inventors have discovered that the AIB1 protein (Amplified In Breast Cancer-1) is a member of the Steroid Receptor Coactivator - 1 (SRC-1) family of nuclear receptor co-activators that interacts with estrogen receptors (ER) to enhance ER-dependent transcription. The inventors have further

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overexpression results in increased ER-dependent transcriptional activity which confers a growth advantage upon AIB1 amplification-bearing clones during the development and progression of estrogen-dependent cancers.

5       Compounds which inhibit or disrupt the interaction of an AIB1 gene product with a steroid hormone receptor, e.g., ER, are useful as anti-neoplastic agents for the treatment of patients suffering from steroid hormone-responsive cancers such as breast cancer, ovarian cancer, prostate cancer, and colon cancer.

10       AIB1 polypeptides or peptide mimetics of such polypeptides, e.g., those containing domains which interact with steroid hormone receptors, can be administered to patients to block the interaction of endogenous intracellular AIB1 and a steroid hormone receptor, e.g., ER in an aberrantly proliferating cell. It is likely that AIB1 interacts with a wide range of human transcriptional factors and that regulation of such interactions will have important therapeutic applications.

15       Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

#### SEQUENCE LISTING

20       The nucleic acid and amino acid sequences listed in the accompanying Sequence Listing are shown using standard letter abbreviations for nucleotide bases and three-letter code for amino acids. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood to be included by any reference to the displayed strand.

[SEQ. I.D. No. 1 shows the nucleic acid sequence of the human AIB1 cDNA and the corresponding amino acid sequence.

25       SEQ. I.D. No. 2 shows the amino acid sequence of the Per/Arnt/Sim (PAS) domain of AIB1.

SEQ. I.D. No. 3 shows the amino acid sequence of the basic helix-loop-helix domain (bHLH) of AIB1.

SEQ. I.D. No. 4 shows the amino acid sequence of the human AIB1 protein.

30       SEQ. I.D. No. 5 shows the nucleic acid sequence of primer N8F1.

SEQ. I.D. No. 6 shows the nucleic acid sequence of the forward primer designed from the 5' sequence of pCMVSPORT-B11, PM-U2.

SEQ. I.D. No. 7 shows the nucleic acid sequence of the reverse primer designed from the 5' sequence of pCMVSPORT-B11, PM-U2.

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SEQ. I.D. No. 8 shows the amino acid sequence of the ER-interacting domain of AIB1.

SEQ. I.D. No. 9 shows the nucleic acid sequence of pCIP, the mouse ortholog of AIB1 and the amino acid sequence for this gene.

5 SEQ. I.D. No. 10 shows the nucleic acid sequence of the forward primer AIB1/mESTF1 used to screen mouse BAC.

SEQ. I.D. No. 11 shows the nucleic acid sequence of the reverse primer AIB1/mESTR1 used to screen mouse BAC.

SEQ. I.D. No. 12 shows the amino acid sequence of pCIP, the mouse ortholog of AIB1.]

10 SEQ ID NO:1 shows the nucleic acid sequence of AIB1 cDNA and the corresponding amino acid sequence.

SEQ ID NO:2 shows the amino acid sequence of the Per/Arnt/Sim (PAS) domain of AIB1.

15 SEQ ID NO:3 shows the amino acid sequence of the basic helix-loop-helix domain (bHLH) of AIB1.

SEQ ID NO:4 shows the amino acid sequence of the human AIB1 protein.

SEQ ID NO:5 shows the nucleic acid sequence of primer N8F1.

SEQ ID NO:6 show the nucleic acid sequence of the forward primer designed from the 5' sequence of pCMVSPORT-B11, PM-U2.

20 SEQ ID NO:7 shows the nucleic acid sequence of the reverse primer designed from the 5' sequence of pCMVSPORT-B11, PM-U2.

SEQ ID NO:8 shows the amino acid sequence of the ER-interacting domain of AIB1.

SEQ ID NO:9 shows the nucleic acid sequence of pCIP, the mouse ortholog of AIB1 and the amino acid sequence for this gene.

25 SEQ ID NO:10 shows the nucleic acid sequence of the forward primer AIB1/mESTF1 used to screen mouse BAC.

SEQ ID NO:11 shows the nucleic acid sequence of the reverse primer AIB1/mESTF1 used to screen mouse BAC.

SEQ ID NO:12 shows the amino acid sequence of pCIP, the mouse ortholog of AIB1.

### 30 FIGURES

[Fig. 1A is a diagram of an amino acid sequence of full length AIB1 in which residues highlighted in black are identical in AIB1, TIF2 and SRC1. Residues identical with TIF2 (GenBank accession number X97674) or SRC-1 (GenBank accession number U59302) are highlighted in grey or boxed, respectively.]

35 Fig. 1A is a diagram of an amino acid sequence of full length AIB1 (SEQ ID NO:4) in which residues highlighted in black are identical in AIB1, TIF2, and SRC1. Residues identical with TIF2 (GenBank Accession No. X97674) or SRC-1 (GenBank Accession No. U59302) are highlighted in grey or boxed, respectively.

40 Fig. 1B is a diagram showing the structural features of AIB1. The following domains are indicated: bHLH domain, PAS domains (with the highly conserved PAS A and B regions shown in

dark gray), S/T (serine/threonine)-rich regions, and a group of charged residues (+/-). A glutamine-rich region and polyglutamine tract are also indicated. The numbers beneath the diagram indicate the location (approximate residue number) of the domain with respect to the amino acid sequence shown in Fig. 1A. The alignment was generated using DNASTAR software.

5 Fig. 2 is a photograph of a Northern blot analysis showing increased expression of AIB1 in the cell lines BT-474, ZR-75-1, MCF7, and BG-1.

Fig. 3 is a bar graph showing that the addition of full length AIB1 DNA to a cell resulted in an increase of estrogen-dependent transcription from an ER reporter plasmid. COS-1 cells were transiently transfected with 250 ng ER expression vector (pHEGO-hyg), 10 ng of luciferase  
10 reporter plasmid (pGL3.luc.3ERE or 10 ng pGL3 lacking ERE) and increasing amounts of pcDNA3.1-AIB1 and incubated in the absence (open bars) or presence of 10 nM 17 $\beta$ -estradiol (E2, solid bars) or 100 nM 4-hydroxytamoxifen (hatched bars). Luciferase activity was expressed in relative luminescence units (RLU). The data are the mean of three determinations from one of four replicate experiments. Error bars indicate one standard deviation.

15 Fig. 4 is a schematic diagram comparing the DNA and protein structures of pCIP (the mouse ortholog of AIB1) and the human AIB1; exons are shown as black boxes.

[Fig. 5 is a table showing the introns and exons of the mouse AIB1 gene (pCIP). The "Exon" column refers to the number of the exon; "cDNA bp 5'-exon" refers to the nucleotide position in the mouse cDNA sequence for the 5' exon. "3' intron splice cite" refers to the last few  
20 nucleotides of the 3' position of the intron. "Exon sequence" refers to the exon itself. "5' intron" refers to the adjacent intron reading from the exon into the splice donor elinucleotides (usually GT).]

Fig. 5 is a table showing the introns and exons of the mouse AIB1 gene (pCIP) (SEQ ID NO:9). The "Exon" column refers to the number of the exon; "cDNA bp 5'-exon" refers to the  
25 nucleotide position in the mouse cDNA sequence for the 5' exon; "cDNA bp 3' exon" refers to the last few nucleotides of the 3' position of the intron. "Exon sequence" refers to the exon itself. "5' intron" refers to the adjacent intron reading from the exon into the splice donor elinucleotides (usually GT).

[Fig. 6 is a table showing the introns and exons of the human AIB1 gene. The "Exon" column refers to the number of the exon; "cDNA bp 5'-exon" refers to the nucleotide position in the  
30 mouse cDNA sequence for the 5' exon. "3' intron splice cite" refers to the last few nucleotides of the 3' position of the intron. "Exon sequence" refers to the exon itself. "5' intron" refers to the adjacent intron reading from the exon into the splice donor nucleotides (usually GT).]

Fig. 6A and Fig. 6B are a table showing the introns and exons of the human AIB1 gene (SEQ ID NO:1). The "Exon" column refers to the number of the exon; "cDNA bp 5'-exon" refers  
35 to the nucleotide position in the mouse cDNA sequence for the 5' exon; "cDNA bp 3' exon" refers to the last few nucleotides of the 3' position of the intron. "Exon sequence" refers to the exon itself. "5' intron" refers to the adjacent intron reading from the exon into the splice donor elinucleotides (usually GT).